Circulating Endothelial Cells From Septic Shock Patients Convert to Fibroblasts Are Associated With the Resuscitation Fluid Dose and Are Biomarkers for Survival Prediction

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Objectives: To determine whether circulating endothelial cells from septic shock patients and from nonseptic shock patients are transformed in activated fibroblast by changing the expression level of endothelial and fibrotic proteins, whether the level of the protein expression change is associated with the amount of administered resuscitation fluid, and whether this circulating endothelial cell protein expression change is a biomarker to predict sepsis survival.

Design: Prospective study.

Setting: Medical-surgical ICUs in a tertiary care hospital.

Patients: Forty-three patients admitted in ICU and 22 healthy volunteers.

Interventions: None.

Measurements and Main Results: Circulating mature endothelial cells and circulating endothelial progenitor cells from septic shock and nonseptic shock patients showed evidence of endothelial fibrosis by changing the endothelial protein expression pattern. The endothelial proteins were downregulated, whereas fibroblast-specific markers were increased. The magnitude of the expression change in endothelial and fibrotic proteins was higher in the septic shock nonsurvivors patients but not in nonseptic shock. Interestingly, the decrease in the endothelial protein expression was correlated with the administered resuscitation fluid better than the Acute Physiology and Chronic Health Evaluation II and Sequential Organ Failure Assessment scores in the septic shock nonsurvivors patients but not in nonseptic shock. Notably, the significant difference between endothelial and fibrotic protein expression indicated a nonsurvival outcome in septic shock but not in nonseptic shock patients. Remarkably, area under the receiver operating characteristic curve analysis showed that endothelial protein expression levels predicted the survival outcome better than the Acute Physiology and Chronic Health Evaluation II and Sequential Organ Failure Assessment scores in septic shock but not in nonseptic shock patients.

Conclusions: Circulating endothelial cells from septic shock patients are acutely converted into fibroblasts. Endothelial and fibrotic protein expression level are associated with resuscitation fluid administration magnitude and can be used as biomarkers for an early survival diagnosis of sepsis. (*Crit Care Med* 2019; XX:00–00)

Key Words: fibrosis; intensive care unit; resuscitation fluid; sepsis; survivor

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Sepsis is a leading source of mortality worldwide in patients admitted to an ICU (1, 2). During septic shock, several alterations are observed, such as hypotension, extensive edema formation, and multiple organ dysfunction syndrome (MODS) (3, 4). Many studies have suggested that increased vascular permeability is a primary factor in the pathogenesis of sepsis-induced MODS and contributes to death (4, 5).

Vascular endothelial monolayer is maintained by the endothelial adhesion proteins, vascular endothelial (VE)-cadherin and CD31, that bind endothelial cells (ECs) to each other preventing vascular leaks (3–5). Decreased VE-cadherin and CD31 expression generate gaps between ECs, leading to increased endothelial permeability.

ECs undergo fibrotic conversion to fibroblasts upon exposure to a wide range of inflammatory mediators through endothelialtomesenchymal transition (EndMT) (6–9). Through EndMT, the endothelial proteins, VE-cadherin and CD31, are downregulated, whereas fibroblast-specific markers, α-smooth muscle actin (α-SMA), and vimentin are upregulated (6–13). However, whether the EndMT-mediated protein expression change participates in the increased vascular permeability and decreased survival of septic shock patients is unknown.

Interestingly, septic patients exhibit an increased number of circulating ECs (CECs) (14–17). Also, CECs are increased in several inflammatory diseases (18–21). Increased number of CEC in septic patients has clinical significance because CECs count has been correlated with survival decreasing (22). CECs are formed by circulating mature ECs (CMECs) and circulating endothelial progenitor cells (CEPCs) (16, 17). Participation of CECs during sepsis are unknown.

Therefore, our aim was to determine whether CECs from patients suffering septic shock exhibited endothelial fibrosis, whether this protein expression change was associated with the amount of administered resuscitation fluid as a measure of increased vascular permeability and whether this CEC expression change could be used as a biomarker to predict sepsis survival.

Our results showed that CMECs and CEPCs from septic shock patients convert to fibroblasts, which are associated with the resuscitation fluid administration magnitude and can be used as biomarkers for an early survival diagnosis of sepsis better than the Acute Physiology and Chronic Health Evaluation (APACHE) II and Sequential Organ Failure Assessment (SOFA) scores.

METHODS

Detailed methods are provided in the **supplemental information** (Supplemental Digital Content 1, http://links.lww.com/CCM/E535).

Study Design

A prospective study was conducted from 2015 to 2018 in patients admitted to the ICU with septic shock or shock without infection at two hospitals in Santiago, Chile (Hospital Clínico Metropolitano La Florida and Hospital Clínico de la Fuerza Aérea). This study was approved by the local institutional Ethics and Bioethics Review Board. The investigation conforms to the principles outlined in the Declaration of Helsinki. The Commission of Bioethics and Biosafety of Universidad Andres Bello also approved all experimental protocols. All participants or their surrogates signed an informed consent form prior to entry into the study.

Group I: critically ill patients admitted to the ICU with a distributive shock diagnosed with septic shock (septic shock group [SSG]). Group II: critically ill patients admitted to the ICU with a distributive shock in the absence of septic shock or infection (non-SSG [NSSG]). This group was principally constituted by following types of patients: neurocritical, acute pancreatitis, and postoperative vascular surgery. Group III: healthy volunteers. See supplemental information (Supplemental Digital Content 1, http://links.lww.com/CCM/E535) for inclusion and exclusion criteria and septic shock definition used.

The septic shock criteria were defined according to the Surviving Sepsis Campaign (4, 23). At the time we planned this study, the current sepsis-3 consensus had not been published (24, 25). Systemic inflammatory response syndrome was defined according to traditional criteria previously published by Bone (26). Resuscitation fluid therapy was only based on crystalloids (ringer lactate and 0.9% saline) and 20% albumin. Twenty-eight-day mortality was also recorded.

CMECs and CEPCs Collection, Isolation, and Analysis

CMECs and CEPCs were obtained from blood samples from SSG and NSSG groups and from healthy volunteers. Collection of blood samples and isolation of cells and their analysis were carried out by double-blinded personnel. The CMECs and CEPCs were isolated as described previously (27, 28) and in the supplemental information (Supplemental Digital Content 1, http://links.lww.com/CCM/E535). Flow cytometry analysis was performed to determine changes in endothelial and fibrotic protein expression.

Data Analysis

The study sample was selected to identify the magnitude effect of a 20% reduction in VE-cadherin expression in ECs between the healthy volunteer group and the SSG with SDs of 10% and 25%, respectively. According to these assumptions, a sample size of 26 patients in the SSG and 20 healthy volunteers would provide 90% statistical power to detect a reduction of VE-cadherin expression of 90% to 75% using a two-sided 0.05 significance level. Categorical variables were presented as proportions and analyzed using the chi-square test. Continuous variables, which were not normally distributed, were described as medians and interquartile ranges (Q3–Q1). The Shapiro-Wilk test was used to test the normality distribution, with p values of greater than 0.10 indicating a normal distribution. Comparisons of continuous variables between two groups were conducted with the t test or the Mann-Whitney U test as appropriate. The relationships between the changes in endothelial protein expression (dependent variable) and the resuscitation fluid or other explanatory variables (independent variables), were subjected to correlation analysis using Spearman correlation coefficients and linear regression.

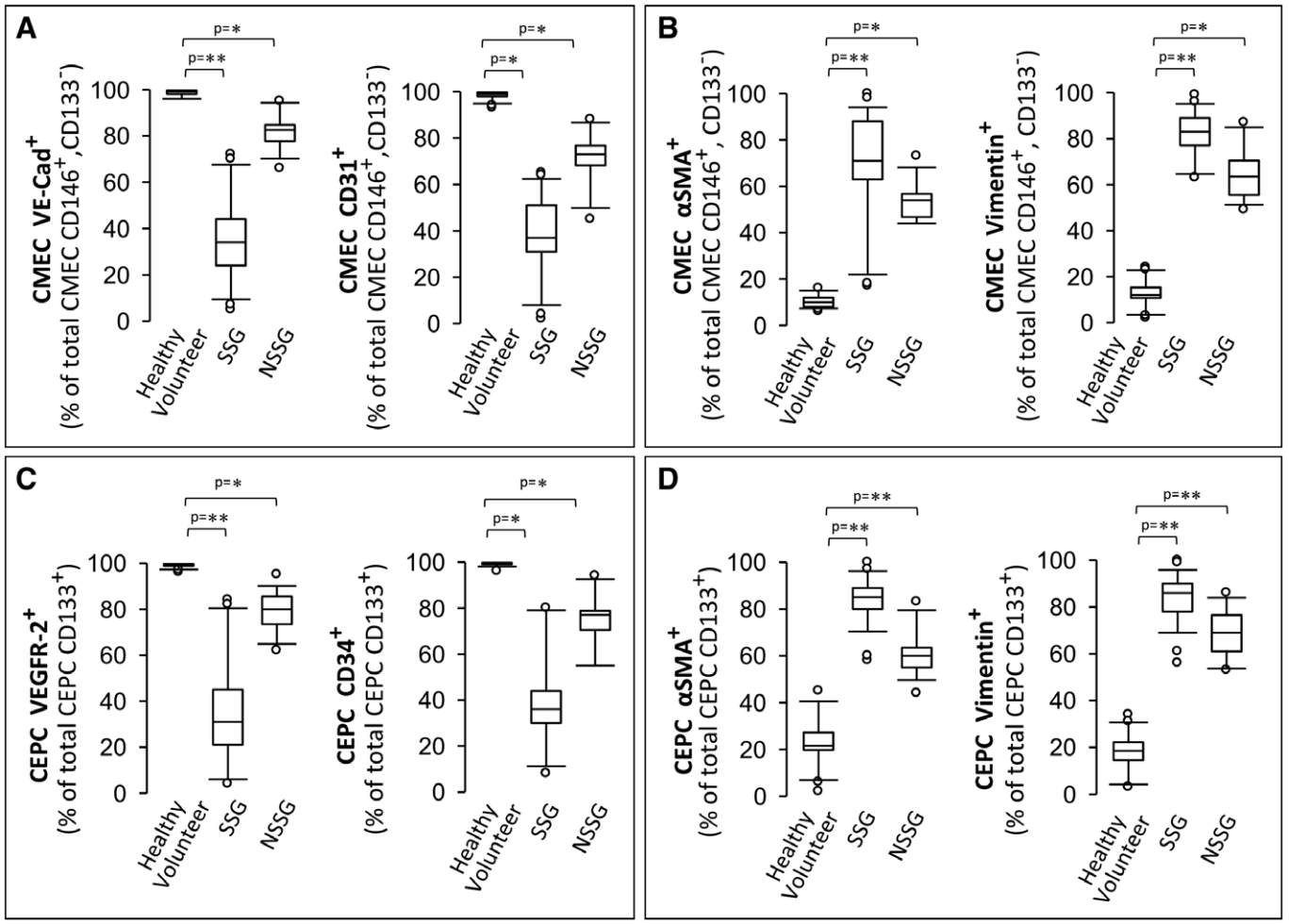

Figure 1. Endothelial and fibrotic proteins expression changes in circulating mature endothelial cells (CMECs) and circulating endothelial progenitor cells (CEPCs) from septic shock group (SSG) and nonseptic shock group (NSSG) patients. Protein expression of vascular endothelial cadherin (VE-Cad$^+$) (**A**, *left*), and CD31$^+$ (**A**, *right*) from CMECs, and vascular endothelial growth factor receptor-2 (VEGFR-2$^+$) (**C**, *left*), and CD34$^+$ (**C**, *right*), from CEPCs, α-smooth muscle actin (α-SMA$^+$) (**B** and **D**, *left*), and Vimentin$^+$ (**B** and **D**, *right*) from CMECs and CEPCs, respectively, were measured in SSG, NSSG, and healthy volunteers conditions. The data are expressed as percentage of total cells counted. Statistical differences were assessed by one-way analysis of variance (Kruskal-Wallis), followed by Dunn's post hoc test. $^*p < 0.05$, $^{**}p < 0.01$. Data are showed as *box plot* indicating median ± interquartile range.

The ability of endothelial and fibrotic protein expression to predict death at 28 days was assessed using the area under the receiver operating characteristic (AUROC) curve (29) with a 95% CI. Statistical testing was two-sided and used the 5% significance level.

RESULTS

CMECs and CEPCs Are Increased in SSG and NSSG Patients

CMECs and CEPCs were isolated from blood samples of SSG and NSSG patients and healthy volunteers. A total of 43 patients (27 SSG and 16 NSSG) and 22 healthy volunteers were enrolled. **Supplemental Table S1** (Supplemental Digital Content 1, http://links.lww.com/CCM/E535) shows the demographic characteristics and clinical data from patients. **Supplemental Table S2** (Supplemental Digital Content 1, http://links.lww.com/CCM/E535) shows the biochemical analysis from patients.

SSG characteristics are shown in **Supplemental Table S3** (Supplemental Digital Content 1, http://links.lww.com/CCM/E535). APACHE II score for SSG and NSSG was 24 (20–25) and 25 (23–27), respectively. SSG and NSSG received a high dose of noradrenaline, had substantial hyperlactatemia, and raised C-reactive protein. The NSSG consists of three types of patients: neurocritical, acute pancreatitis, and postoperative vascular surgery. NSSG characteristics are shown in **Supplemental Table S4** (Supplemental Digital Content 1, http://links.lww.com/CCM/E535). Mortality was 37.0% and 43.8% for SSG and NSSG, respectively ($p = 0.663$).

CMECs (CD146$^+$, CD133$^-$) and CEPC (CD133$^+$) were higher in both the SSG and NSSG compared with healthy volunteers (14, 15). (**Supplemental Fig. S1**, ***A*** and ***B***, Supplemental Digital Content 1, http://links.lww.com/CCM/E535). Total population of CECs was increased in SSG and NSSG (**Supplemental Fig. S1*C***, Supplemental Digital Content 1, http://links.lww.com/CCM/E535).

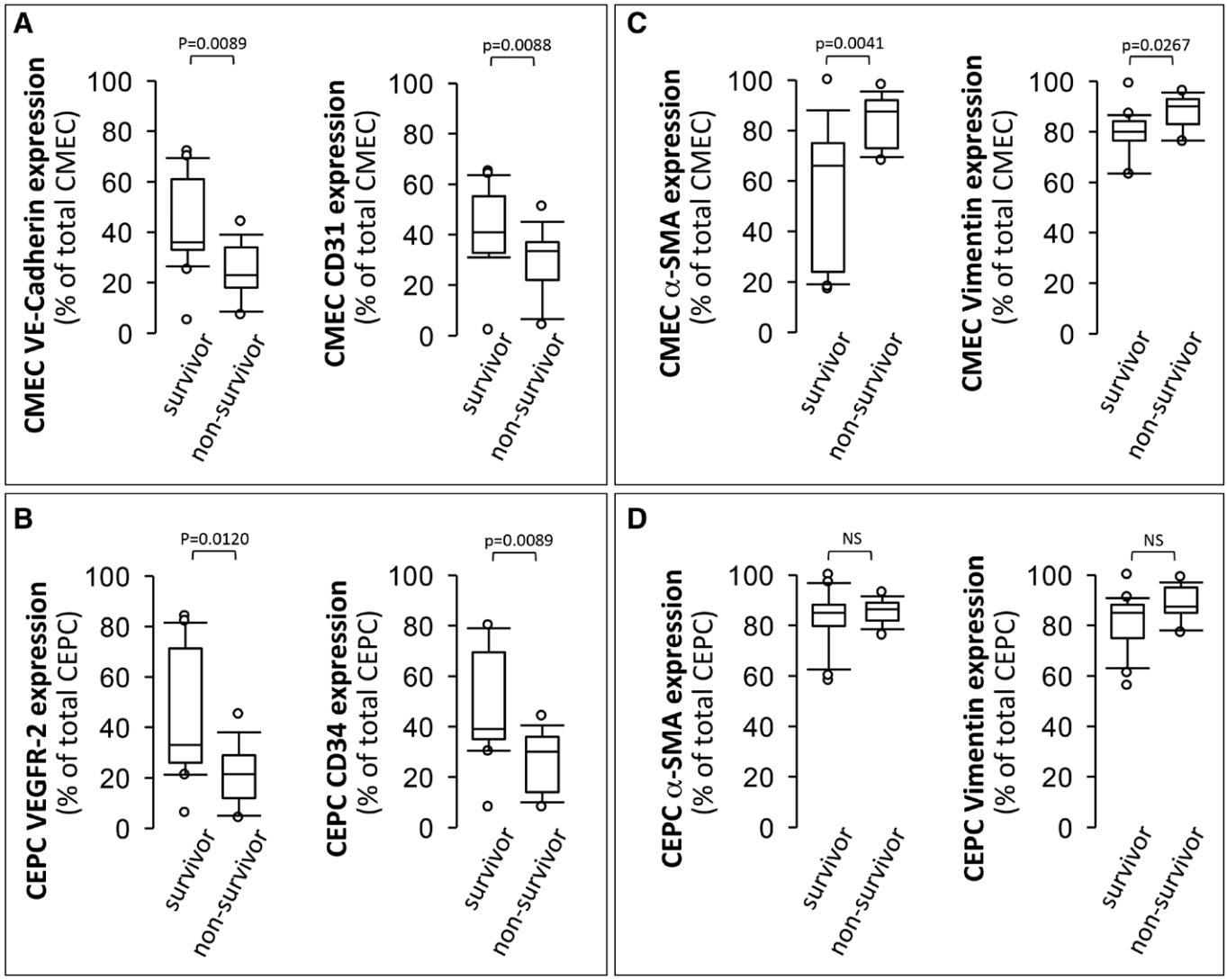

Figure 2. Endothelial and fibrotic markers expression changes in circulating mature endothelial cells (CMECs) and circulating endothelial progenitor cells (CEPCs) is different between survivor and nonsurvivor groups from septic shock group (SSG) patients. Protein expression of vascular endothelial cadherin (VE-cadherin) and CD31 (**A**), and α-smooth muscle actin (α-SMA) and Vimentin (**C**), from CMECs, and vascular endothelial growth factor receptor-2 (VEGFR-2) and CD34 (**B**) and α-SMA and Vimentin (**D**), from CEPCs, in survivors and nonsurvivors patients in SSG, were tested. The data are expressed as a percentage of total cells counted. Statistical differences were assessed by Student *t* test (Mann-Whitney *U* test). Data are showed as *box plot* indicating median ± interquartile range. NS = non significant.

Endothelial Protein Expression Is Decreased and Fibroblast Marker Expression Is Increased, in CMECs and CEPCs From SSG and NSSG Patients

VE-cadherin and CD31 expression in CMECs ($CD146^+$, $CD133^-$) decreased in both the SSG and NSSG patients compared with those in healthy volunteers (**Fig. 1*A***). Expression of the endothelial progenitor markers vascular endothelial growth factor receptor-2 (VEGFR-2) and CD34 in the CEPCs ($CD133^+$) was lower in SSG and NSSG patients (**Fig. 1*C***). A significant proportion of CMECs ($CD146^+$, $CD133^-$) from SSG and NSSG blood samples expressed the fibrotic markers α-SMA and vimentin (**Fig. 1*B***). CEPCs ($CD133^+$) from SSG and NSSG blood samples exhibited a large increase in expression of the fibrotic markers α-SMA and vimentin (**Fig. 1*D***). In accordance, fluorescent immunocytochemistry shown concordant results (**Supplemental Fig. S2**, Supplemental Digital Content 1, http://links.lww.com/CCM/E535). Interestingly, CMECs and CEPCs cells from healthy volunteer incubated with endotoxin showed a strong change in the expression pattern that indicates endothelial conversion into fibroblast (**Supplemental Fig. S3**, Supplemental Digital Content 1, http://links.lww.com/CCM/E535). Similarly, CMECs and CEPCs cells from healthy volunteer incubated with transforming growth factor-β showed also endothelial fibrosis (**Supplemental Fig. S4**, Supplemental Digital Content 1, http://links.lww.com/CCM/E535).

Changes in Endothelial and Fibrotic Marker Expression in CMECs and CEPCs Differ Between the Survivor and Nonsurvivor Groups of SSG Patients

Although Figure 1 showed that endothelial markers were decreased and fibrotic markers were increased in SSG and NSSG, we examined whether the magnitude of the changes in

endothelial and fibrotic marker expression in the CMECs and CEPCs was different between the survivor and nonsurvivor groups of SSG and NSSG patients. To that end, we compared the expression of each endothelial and fibrotic marker between the survivor and nonsurvivor groups from the SSG and NSSG conditions. The results obtained in SSG patients showed that VE-cadherin and CD31 expression in CMECs decreased more significantly in the nonsurvivor groups (**Fig. 2*A***). Similarly, VEGFR-2 and CD34 expression in CEPCs decreased more significantly in the nonsurvivor groups (**Fig. 2*B***). The fibrotic markers α-SMA and vimentin increased more in the CMECs from the nonsurvivor groups (**Fig. 2*C***), whereas the same fibrotic proteins showed no differences in expression in the CEPCs (**Fig. 2*D***). Analyses performed in NSSG patients showed no differences in protein expression between the survivor and nonsurvivor groups (**Supplemental Fig. S5**, Supplemental Digital Content 1, http://links.lww.com/CCM/E535).

Endothelial and Fibrotic Protein Expression Changes in CMECs and CEPCs From SSG Patients Correlate With an Increase in the Administered Resuscitation Fluid

In the SSG patients, decreased VE-cadherin and CD31 expression in the CMECs were correlated with an increase in the administered resuscitation fluid, which was used as an indirect measure of the magnitude of vascular permeability (**Fig. 3*A***). A similar analysis performed in the CEPC population showed that decreased VEGFR-2 and CD34 expression correlated with the increase in the administered resuscitation fluid (**Fig. 3*B***).

Furthermore, increased expression of α-SMA and vimentin in the CMECs were correlated with the administered resuscitation fluid increase (**Fig. 3*C***). In contrast, the evaluation in CEPCs showed that increased expression of α-SMA was not correlated (but vimentin correlated with low significance) with an increase in the administered resuscitation fluid (**Fig. 3*D***). Highlighting the importance of the correlation between the resuscitation fluid and the endothelial and fibrotic markers, **Supplemental Table S5** (Supplemental Digital Content 1, http://links.lww.com/CCM/E535) shows the low correlation with multiple other clinical variables.

Notably, very low correlation was observed between the administered resuscitation fluid and the APACHE II and SOFA score (**Fig. 3**, ***E*** and ***F***), discarding the hypothesis regarding that the high correlation between the amount of resuscitation fluid and the endothelial and fibrotic markers expression was

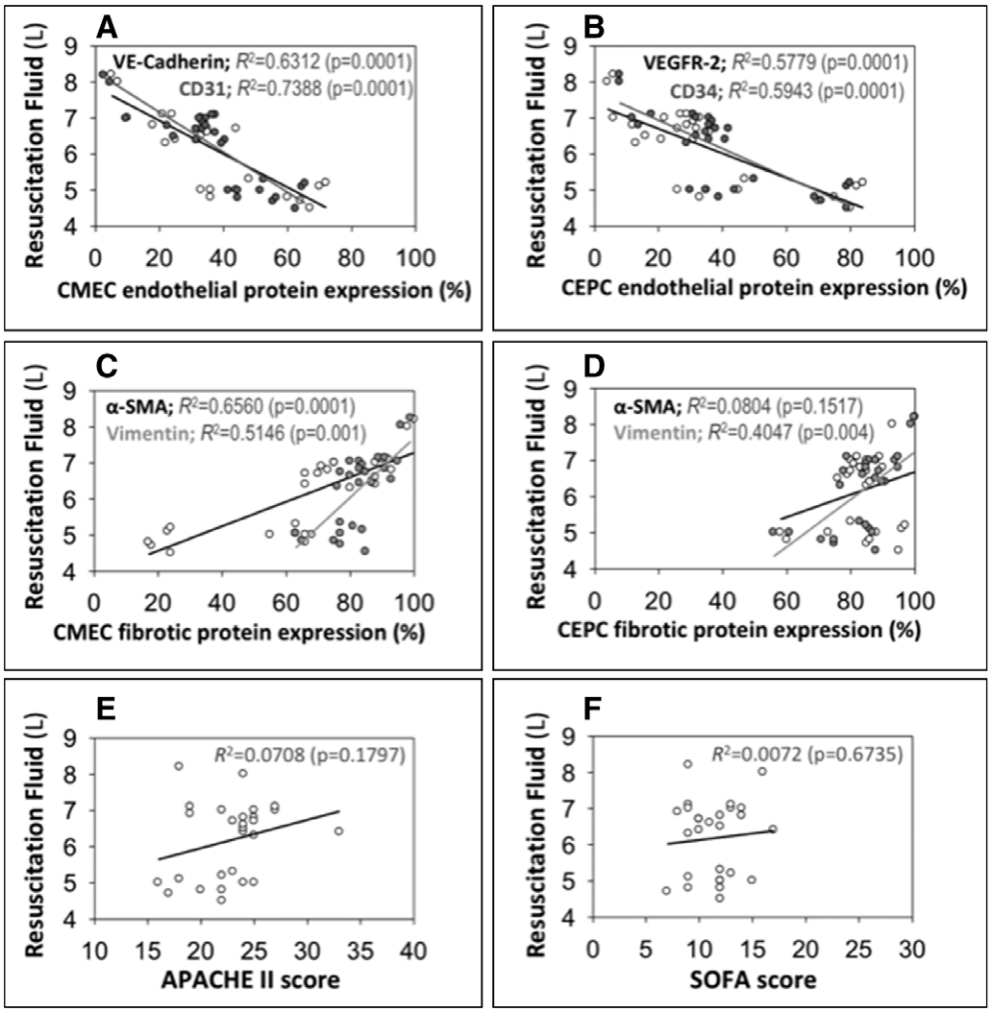

Figure 3. Endothelial and fibrotic protein expression changes correlate with the increase in the resuscitation fluid administration in circulating mature endothelial cells (CMECs) and circulating endothelial progenitor cells (CEPCs) from septic shock group (SSG) patients. Correlation analyses between endothelial (**A** and **B**) and fibrotic (**C** and **D**) markers expression change, and the Acute Physiology and Chronic Health Evaluation (APACHE) II score (**E**) and Sequential Organ Failure Assessment (SOFA) (**F**) score, with the resuscitation fluid administration required in the first 24 hr after admission to ICU, in CMECs (**A** and **C**) and CEPCs (**B** and **D**) in SSG. Correlation between resuscitation fluid and the following protein expression: vascular endothelial cadherin (VE-cadherin) expression ([**A**]; $R^2 = 0.6312$, $R = 0.7944$, $p = 0.0001$), CD31 ([**A**]; $R^2 = 0.7388$, $R = 0.8595$, $p = 0.0001$), α-smooth muscle actin (α-SMA) ([**C**]; $R^2 = 0.6560$, $R = -0.8099$, $p = 0.0001$), and vimentin ([**C**]; $R^2 = 0.5146$, $R = -0.7173$, $p = 0.001$) in CMECs, and vascular endothelial growth factor receptor-2 (VEGFR-2) ([**B**]; $R^2 = 0.5779$, $R = 0.7601$, $p = 0.0001$) and CD34 ([**B**], $R^2 = 0.5943$, $R = 0.7709$, $p = 0.0001$), α-SMA ([**D**], $R^2 = 0.0804$, $R = -0.2835$, $p = 0.1517$), and vimentin ([**D**]; $R^2 = 0.4047$, $R = -0.6361$, $p = 0.004$), in CEPCs, were tested. Correlation between resuscitation fluid and APACHE II score ([**E**]; $R^2 = 0.0708$, $R = -0.2660$, $p = 0.1797$) and SOFA score ([**F**]; $R^2 = 0.0072$, $R = -0.0848$, $p = 0.6735$). The coefficient of correlation (R^2) and p value were determined by linear regression. We considered correlation when R^2 and p values were $R^2 > 0.400$ and $p < 0.01$.

a consequence of the severity of septic shock. No differences were observed in NSSG (**Supplemental Fig. S6**, Supplemental Digital Content 1, http://links.lww.com/CCM/E535).

Endothelial and Fibrotic Markers Expression Change Comparison in CMECs and CEPCs Determine Survivor and Nonsurvivor Outcome in SSG Patients

Noteworthy, expression level comparison between the endothelial marker VE-cadherin and the fibrotic markers α-SMA and vimentin, in CMECs from SSG patients (**Fig. 4*A***), showed significant differences in the nonsurvivor groups, whereas comparison performed in survivor groups were not different. Similarly, CD31 expression level compared with α-SMA and vimentin, in CMECs from SSG patients (**Fig. 4*B***), showed significant differences in the nonsurvivor groups, whereas no differences were detected in survivor groups. VEGFR-2 expression level compared with α-SMA and vimentin in CEPCs from SSG patients (**Fig. 4*C***), showed significant differences in the nonsurvivor groups, whereas comparison performed in survivor groups were not different. Similarly, CD34 expression level compared with α-SMA and vimentin, in CEPCs from SSG patients (**Fig. 4*D***), showed significant differences in the nonsurvivor groups, whereas no differences were detected in survivor groups. Similar analyses performed in NSSG patients did not show any difference (**Supplemental Fig. S7**, Supplemental Digital Content 1, http://links.lww.com/CCM/E535).

Endothelial Protein Expression Levels As Biomarkers for Predicting Survival in SSG Patients

To quantify the capacity to predict survival through measuring the expression pattern of endothelial and fibrotic proteins from CMECs and CEPCs in SSG patients, we performed an AUROC curve analysis. We focused on three related issues in this study: 1) evaluation of the capacity of each single endothelial or fibrotic protein to predict survival in SSG patients; 2) determination of the best cut-off value in terms of the sensitivity and specificity of each single endothelial or fibrotic protein for the accurate prediction of survival in SSG patients; and 3) comparison of the

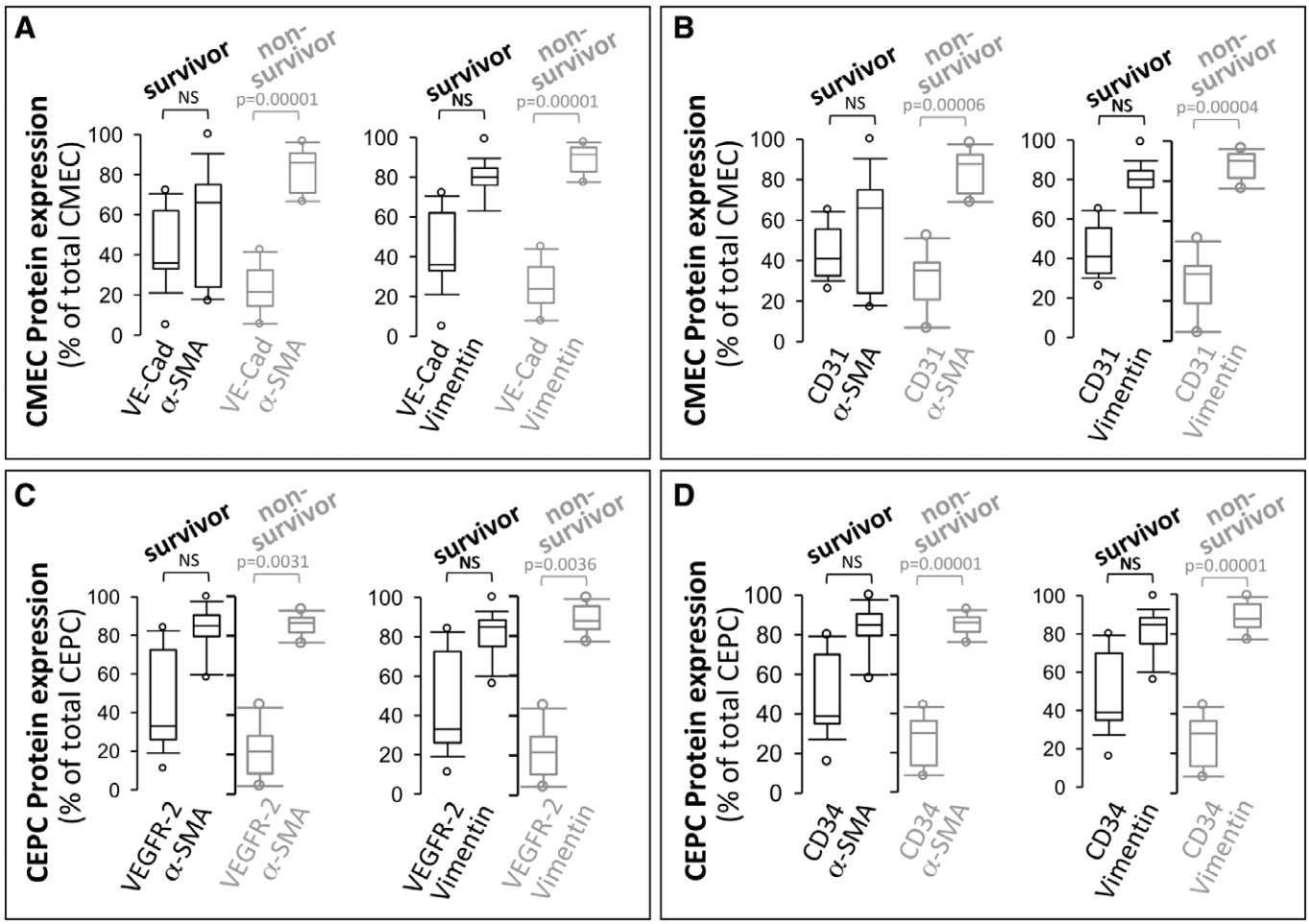

Figure 4. Endothelial and fibrotic markers expression change comparison in circulating mature endothelial cells (CMECs) and circulating endothelial progenitor cells (CEPCs), as biomarkers to determine survivor and nonsurvivor outcome in septic shock group (SSG) patients. Protein expression of vascular endothelial cadherin (VE-cadherin) versus α-smooth muscle actin (α-SMA) (**A**, *left*), VE-cadherin versus vimentin (**A**, *right*), CD31 versus α-SMA (**B**, *left*), CD31 versus vimentin (**B**, *right*), from CMECs, and vascular endothelial growth factor receptor-2 (VEGFR-2) versus α-SMA (**C**, *left*), VEGFR-2 versus vimentin (**C**, *right*), CD34 versus α-SMA (**D**, *left*), CD34 versus vimentin (**D**, *right*), from CEPCs, in survivors and nonsurvivors patients in SSG, were tested. The data are expressed as a percentage of total cells counted. Statistical differences were assessed by Student *t* test (Mann-Whitney *U* test). Data are showed as *box plot* indicating median ± interquartile range. NS = non significant.

capacity of each single endothelial or fibrotic protein to predict survival versus two scores of severity and prognosis used for critical patients (APACHE II and SOFA).

Endothelial proteins from the CMECs and CEPCs showed a high predictive capacity (**Fig. 5**, ***A*** and ***B***) with AUROC values greater than 0.73 and 95% CIs that left out the nondiscrimination line (diagonal discontinuous line in plots), which supported their statistical significance. None of the fibrotic markers surpassed the nondiscrimination line (**Fig. 5**, ***C*** and ***D***); thus, they do not have predictive capacity to discriminate the survival outcome in SSG patients. The best sensitivity and specificity to predict the survival outcome in SSG patients in addition to the Youden index (YI) (30) for each endothelial protein were as follows: VE-cadherin: sensitivity: 64.7%, specificity: 90%, YI: 0.54, cut-off ≥ 35%; CD31: sensitivity: 58.8%, specificity: 90%, YI: 0.48, cut-off ≥ 40%; VEGFR-2: sensitivity: 64.7%, specificity: 90%, YI: 0.55, cut-off ≥ 32%; and CD34: sensitivity: 58.8%, specificity: 90%, YI: 0.49, cut-off ≥ 38%. Because fibrotic proteins do not surpass the nondiscrimination line, they do not discriminate the survival outcome in SSG patients. Capacity to predict survival outcomes based on the APACHE II and SOFA scores was poor compared with that of the endothelial proteins (Fig. 5, *A* and *B*). AUROC values for APACHE II and SOFA scores were 0.31 (95% CI, 0.1–0.52) and 0.37 (95% CI, 0.13–0.62), respectively. Furthermore, the best sensitivity and specificity values and YIs for prediction of the survival outcome were as follows: APACHE II: sensitivity 76.5%, specificity 20%, YI –0035, cut-off ≥ 20 and SOFA: sensitivity 70.6%, specificity 30%, YI 0.006, cut-off ≥ 10. AUROC analyses performed in NSSG patients showed a low predictive capacity (**Supplemental Fig. S8**, Supplemental Digital Content 1, http://links.lww.com/CCM/E535).

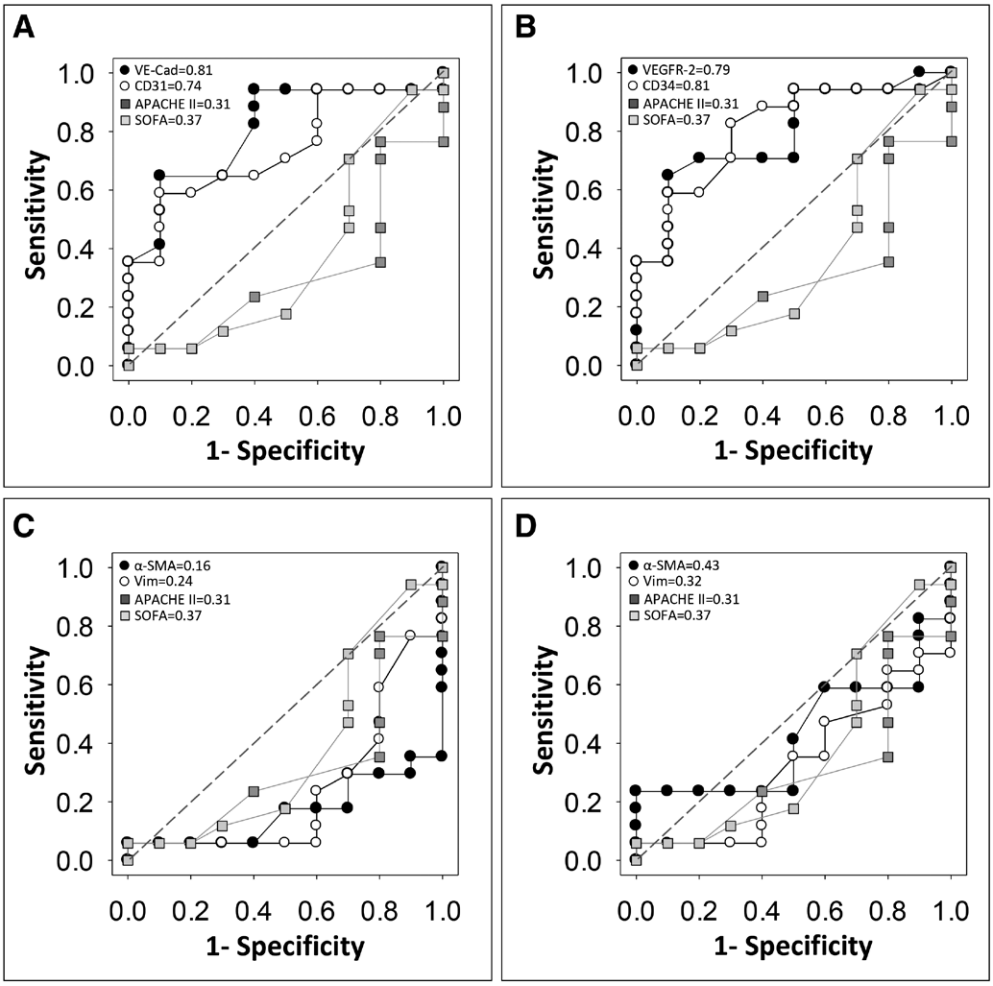

Figure 5. Endothelial protein expression level as biomarkers for predictive surviving in septic shock group (SSG) patients. Area under the receiver operating characteristic curve analysis of expression pattern of endothelial and fibrotic proteins from circulating mature endothelial cell (CMEC) and circulating endothelial progenitor cell (CEPC) in SSG were performed for vascular endothelial cadherin (VE-cadherin): 0.81 (95% CI, 0.63–0.98) and CD31: 0.74 (95% CI, 0.55–0.93) in CMEC (**A**). Vascular endothelial growth factor receptor-2 (VEGFR-2): 0.79 (95% CI, 0.62–0.97) and CD34: 0.81 (95% CI, 0.63–0.98) in CEPC (**B**). α-smooth muscle actin (α-SMA): 0.16 (95% CI: 0.63–0.98) and vimentin (Vim): 0.24 (95% CI, 0.55–0.93) in CMEC (**C**). α-SMA: 0.43 (95% CI, 0.62–0.97) and vimentin: 0.32 (95% CI, 0.63–0.98) in CEPC (**D**). Diagonal discontinuous line in plots is the nondiscrimination line. APACHE = Acute Physiology and Chronic Health Evaluation, SOFA = Sequential Organ Failure Assessment.

DISCUSSION

Septic patients show progressive tissue and organ edema, suggesting a widespread increase in vascular permeability (31, 32). Edema formation could contribute to MODS. Thus, a reduction of edema is a well-recognized factor for sepsis recovery, which is in line with the restoration of vascular permeability (33). VE-cadherin and CD31 are key for endothelial cell-to-cell connections, attaching adjacent ECs and prevents vascular leakage (34). On the other hand, the acquisition of fibrotic proteins possibly generates a cytoskeletal rearrangement that changes the cell structure and promotes cell detachment similar to demonstrations in in vitro cultured EC exposed to endotoxic conditions (7, 8). Thus, the correlation observed between the decrease in endothelial protein expression and the increase in the administered resuscitation fluid suggested an association between these phenomena that was supported by a coherent biological explanation.

Multiple lines of clinical evidence have indicated that vascular hyperpermeability plays a crucial role in the outcome of sepsis (35). Signs of

hypoperfusion, which increased vascular permeability could contribute to this issue (3–5). Because low arterial pressure is a main feature of sepsis syndrome, a general strategy in sepsis treatment is to administer a resuscitation fluid to achieve adequate perfusion. Despite this approach, intravascular administration of high resuscitation fluids often fails to restore perfusion (3–5).

Our results surprised us by showing that the expression levels of endothelial proteins in CMECs and CEPCs had the ability to predict survival better than the classic scores of gravity and organic failure, such as the APACHE II and SOFA scores, respectively. Our study has several strengths that are in accordance with previously stated recommendations (36), demonstrating that results of the forecasting capacity are sufficiently robust from a methodological perspective. The main strengths are as follows: 1) all patients were followed for 28 days or until death, which is a gold standard in survival allocation; 2) the study design and experimental procedures were performed in a rigorous double-blind manner; thus, the nonmedical personnel who performed and analyzed the protein expression determinations in the CECs did not participate in patient management or the survival evaluation, and the medical researchers did not know the expression levels of the endothelial proteins when evaluating the survival outcomes; and 3) the nonsurvival outcome occurred in a significant proportion of patients with septic shock (37% mortality).

Our study has some limitations regarding the predictive capacity of survival based on endothelial proteins in SSG patients. The SSG was a group of patients undergoing a restricted spectrum of septic shock because they were recruited using strict inclusion and exclusion criteria. At this time, extrapolating this information to a general population of patients undergoing septic shock is not possible. Although our statistics regarding the ROC curves have significance with CIs that do not include the nondiscrimination line, the CIs are rather large.

We previously demonstrated that ECs exposed to endotoxin down-regulate endothelial proteins and up-regulate fibrotic markers (7, 10, 11, 13). Furthermore, ECs exposed to cytokines also exhibited changes in protein expression (6, 8, 9). This evidence would indicate that an inflammatory condition in the absence of infection could induce endothelial reprogramming. However, our results showed that only the septic condition (SSG patients) but not the NSSG patients who exhibited systemic inflammation but not infection were able to 1) both decrease endothelial and increase fibrotic protein expression, 2) correlate with the increment of the administered resuscitation fluid, and 3) show differential significance between endothelial and fibrotic protein expression as a predictor for septic patient survival/nonsurvival outcomes.

Despite the high short-term mortality observed during sepsis, many patients are able to survive the septic episode. Additionally, several studies have indicated that many surviving septic patients exhibit an increased risk of death from nonseptic related causes in the years following their initial admission to the ICU (37, 38). These surviving patients acquire several important diseases or pathologic conditions that are not obviously related to sepsis, such as tumor growth, the appearance of cancer, increased pro-inflammatory cytokine production, immunosuppression, cognitive deficiency, peripheral nerve function impairment, and skeletal muscle disability (39, 40). Sepsis-induced endothelial dysfunction could potentially generate alteration in organ perfusion (41). We previously showed that attached ECs in a human blood vessel perfused with endotoxin exhibits a strong change in the level of endothelial and fibrotic protein generating fibrosis (7) In this context, widespread endothelial fibrosis in attached EC at tissue level could contribute to and/or initiate the pathologic conditions detected in survivor patients several years after the septic condition. Also, using postmortem septic human samples, it would be possible to test whether attach EC from septic patients exhibit fibrosis. However, several well-designed experiments must be performed to demonstrate these ideas.

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